

Attorney Docket No.: DC-0199
Inventors: Cheung et al.
Serial No.: 10/043,539
Filing Date: January 11, 2002
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In the Specification:

Please replace the paragraph beginning at page 24, line 28, with the following rewritten paragraph:

--**Cloning and sequence analysis of the *sarR* gene.** To clone the gene encoding SarR, we blotted the ~12 kDa protein onto a PVDF membrane for N-terminal sequencing. The first 14 amino acids were X(K)IND(I)NDLVNA(S/T)F, (~~Seq-~~ SEQ. ID NO.:8) with X being an unknown residue while those residues in parenthesis carried a putative assignment. In search the databank of the partially released *S. aureus* genome (~~www.tiger.org~~), we obtained a partial ORF of 47 amino acid ~~sequence~~ acids that corresponds to the N-terminal sequence of the ~12 kDa protein. By using two degenerate oligonucleotides of 30-nt each, a 141-bp fragment was amplified to probe a chromosomal digest of *S. aureus* strain RN6390, thus allowing identification of a ~4 kb *Cla*I hybridizing fragment. A plasmid DNA library containing ~3.5 kb *Cla*I fragments constructed in pACYC177 (26) was then screened with the 141-bp PCR-generated probe. A positive clone (pALC1361) yielding a ~4-kb insert at the *Cla*I site of pACYC177 vector was identified. In determining the sequence of the insert, and comparing the insert sequence with that of the 141-bp probe, the DNA sequence of the putative gene *sarR* was obtained (Fig. 1B) (GenBank accession #AF207701). The predicted SarR protein contains 115 amino acids, with a predominance of charged residues (34%) and a predicted molecular size of 13,689 daltons. The *sarR* gene has a putative shine Dalgarno sequence (AGGAGTGG) (SEQ. ID NO:9) lying 7-bp upstream of the translation star, with typical initiation (ATG) and termination codons (TAA).

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To ascertain the transcription start site and the putative promoter boxes, the 5'-end of the *sarR* transcript was mapped by primer extension, using an internal primer of the non-coding strand positioned near the N-terminus of the *sarR* coding region. The transcription initiation site is located 88-bp upstream of the translation start, thereby allowing identification of the putative -10 and -35 promoter boxes as TAGAAT (SEQ ID. ~~No.~~ NO:10) and TTACCG (SEQ ID. ~~No.~~ NO:11), respectively (Fig. 1B).--